**Course Name :An Overview on Maternal Health Antenatal, Intranatal and Postnatal Care**

**Professor Name: Dr. Barnali Ghosh**

**Department Name: Multidisciplinary**

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## Prenatal Screening and Diagnosis of aneuploidies

Hello students. I welcome you all to today's session for the NPTEL online certified course on the topic and overview on maternal health, the antenatal, intranatal and postnatal care. I am Dr. Barnali Ghosh, an obstetrician and gynecologist working as assistant professor at B.C.Roy Medical College and Medical Research Center, IIT Kharagpur. Today our topic of discussion is prenatal diagnosis and screening for aneuploidies. So the concepts to be covered in today's class are the prenatal screening of the different aneuploidies, the tests and the diagnostic markers done for screening of most common genetic disorder prevalent that is the Down syndrome and the various ultrasound markers which are associated with different aneuploidies.

So basically we are trying to screen the high risk group pregnancies who are more prone to aneuploidies and anomalous babies. The keywords are as follows, right. So coming to the discussion, what is the objective? Why are we going to do these prenatal diagnostic tests? It is to evaluate a fetus with the risk of chromosomal or genetic abnormality or a structural abnormality, right. So there can be various abnormality on the part of the fetus.

It can be in the number of chromosomes, in the disposition of chromosomes or it can be a single, right. So chromosomal abnormality there are various types, what are they? It can be trisomy, right. It can be triploidy. It can be monosomy. Different you know names given according to the difference in the number of chromosomes.

Normally in a case of human fetus, normally it is 46 pair, 46 chromosome that means 23 pairs of chromosomes, right out of which now 46xx that means, the fetus is a female, it is a female fetus and 46xy it means a male fetus. So this is regarding the chromosome. Now coming to genetic abnormality, single gene mutation, right. Any single gene point mutation or point deletion can lead to abnormal babies. It can lead to structural or different types of syndromes leading to anomalous babies, right.

So these anomalous babies need to be screened. Different types of abnormalities which I was discussing. It can be chromosomal which can be in the number, different in the number or

different in the structure. Single gene disorder like example is cystic fibrosis, right. It can be polygenic or multifactorial.

What does that mean? That means the different syndromes, Edwards syndrome, these are also chromosomal. Sometimes chromosomal, environmental, genetic, metabolic, all these multifactorial disorders lead to anomalous baby and due to some drug ingestion during the period of pregnancy, due to exposure to exogenous factors or drugs, it can be teratogenic disorders in the fetus like in case of fetal alcohol syndrome. Due to ingestion of alcohol during pregnancy in the first trimester, there can be, you know, flat fetal faces which is called as fetal alcohol syndrome. Various drugs, we will discuss in the subsequent classes, various drugs like valproic acid, lithium, these all cause anomalies in the baby and should not be taken most importantly in the first trimester which is the period of organogenesis, right. So, coming to prenatal screening.

Screening what is it? It is a process of testing a population that is apparently healthy. Apparently, the mother, the father are healthy, but still we need to go for the screening using some specific marker or markers to detect a particular condition, right, to detect some particular chromosomal abnormality or aneuploidy using a predefined cutoff. We have a cutoff and above that cutoff, if the screening test results come above the cutoff, then they are called to be screened positive and they are at risk and so more diagnostic tests should be done in that couple, right. So, using a predefined cutoff, the screening test help to divide the population into high risk and low risk and this high risk group will be offered the diagnostic test or the confirmatory test. Screening test as such is non-invasive mostly by the USG parameters and the blood test, but diagnostic tests are mostly invasive.

There are also some non-invasive test, but invasive test where we extract the fetal cells and then examine the chromosomal, you know, makeup of the fetus. So, prenatal screening is aimed at assessing the risk of chromosomal abnormalities, especially aneuploidy. We will be discussing today the different aneuploidies which are screened in no regular basis in all pregnancies, it should be screened in all pregnancies. So, what is aneuploidy? It is abnormal chromosomal number, right. So, abnormal chromosomal number, what is normal? Normal is 46 chromosome that is 23 pairs of chromosome of which one is sex chromosome, right.

So, 22 pairs of autosome plus one pair of sex chromosome and this sex chromosome can be XX in case of females and XY in case of males, right. So, this is the normal structure and if there is any abnormality in the number of chromosome, it may be more or it may be less, right. So, most commonly trisomy, trisomy meaning of which these are the most common trisomy 13, trisomy 18, trisomy 21. That means, normally there is one pair that is diploid, right. One chromosome 1 has a pair, chromosome 2 has 2 pairs, right.

So, these are pairs the chromosomes are present in the human cell in pairs, but if there are 3 in number say chromosome 21, chromosome 21 is 3 in number. So, this is abnormal, this is chromosome 21, it is trisomy 21, right. So, what are the names? Trisomy 13 is Patau syndrome, trisomy 18 is Edwards syndrome and trisomy 21 which is very very common it is Down syndrome, right. So, there can be trisomy, there can be triploidy, all the chromosome pairs are present in 3 in number all the total set. So, that is called as triploidy.

So, it will be having 69 chromosomes, right and or in case of monosomy, monosomy having only one chromosome of that number say monosomy X. Monosomy X meaning it has 22 pairs of autosomes and only one X chromosome, right also called as X written as X O or X 0, right. So, this is monosomy X and it is known as Turner syndrome. So, these are different types of aneuploidies and now maximum trisomy are present mostly 90 percent of the aneuploidies are trisomy chromosome number 13, 18 and 21 or monosomy X or Y, right because other trisomies or other triploidies they mostly get aborted in the early stages of pregnancy. Now, coming to trisomy 21, it is the most common type of abnormality and there are different types of trisomy 21 or different types of Down syndrome.

Number 1 is non-disjunction, number 2 is translocation and 3 is mosaicism. These are divided based on the cause of this aneuploidy or cause of this trisomy 21 of which non-disjunction, this is the most common. 95 percent of the Down syndrome will result from sporadic chromosomal non-disjunction during the process of meiosis. Say during the process of meiosis the ovum and the sperm should be haploid, but say one of the gametes have 2 21 number chromosome and other gamete is haploid having one number of chromosome 21. So, this is chromosome 21.

Now when these 2 fuse they will have a trisomy 21. This is leading to the formation of trisomy 21 mostly due to non-disjunction during meiosis. So, why are we bothered about this trisomy 21 and why all the tests are you know pointing towards detection of this Down syndrome. Down syndrome is nothing but trisomy 21. Number 1 because the prevalence is very high how much 1 in 800 live births and this is the most common cause of inherited intellectual disability in humans right.

So, 90 percent of the mothers who have these affected fetus are young, young meaning less than 35 years of age. So, even in younger age group mothers are having babies with trisomy 21 and so all pregnancies should be screened. All pregnancies should be screened for this trisomy 21 and say in case there is a previous history of Down syndrome right in the previous pregnancy or there is a previous history of trisomy or any type of anomalous baby in the previous pregnancy then that is a case of high risk pregnancy because there is a risk of recurrence and then we directly go to the diagnostic test. We will not go we will not place the mother for the screening tests, but we will directly ask her to go for the diagnostic test so as to you know detect any anomaly beforehand and if the fetus is detected to have some anomaly in the form of

aneuploidy or in the form of other you know types of chromosomal abnormalities right, genetic defects or cardiac defects then the parents can be counseled regarding termination of pregnancy if they want or can continue the pregnancy as per their wish right. So, thus we can tell them at a very early stage where if abortion is needed it can be done you know in the early trimester within 8 weeks 8 to 11 weeks right.

So, coming to the screening test how a screening test should be it should you know every test it has a positive screening, positive rate, negative rate, positive predictive value and negative predictive value every test has such and we need to have an ideal test right where we can detect more and more persons at risk right. So, if a screening test is positive what does the screen positive rate means if a screening test is positive then you know what percentage is that the female with a screen positive is actually having an anomalous baby. It may happen that even if she is screened as positive her baby is normal right. So, you know positivity rate should be high if she is screened as positive then you know it is expected that her fetus is abnormal, but say the screen positive rate is you know low that means, that even if a patient or a mother is screened positive she has a normal baby. So, this will add to the anxiety of the mother right because the mother will think that her baby may be anomalous and she will be panicking right.

So, she will be put to unnecessary further tests unnecessary further test in the form of diagnostic tests which are not required right. So, these will all add to the burden of the mother as well as from the point of from the point of the doctor right. So, positivity rate should be high, negativity rate should be low, positive predictive value those who are screened as positive what is the chance that they have their babies have the disease right. So, these are all statistical terms and these will define a screening test right. So, what are the screening tests that we are doing? Non-invasive firstly it is non-invasive every screening test should be feasible, acceptable, affordable to the mother and to start with they are non-invasive prenatal screening for aneuploidy or neural tube defects it should be offered to all women regardless of age because it has been seen that even women of lesser age less than 35 years they are also having babies of Down's syndrome.

So, how to go about the screening in the first trimester at around 11 to 13 6 by 7 weeks we will first counsel and tell her to go for the USG with NT scan, Nuchal Translucency Scan. So, this is the USG parameter right and plus some blood test that is the dual marker test right. So, this is the first trimester screening and if this screen is negative that means, there is no high risk. So, it is a normal pregnancy and continue as you know monitor a normal pregnancy, but if this comes out to be positive then there are two approach. Number one approach is a secondary screening test we can go for again a secondary screening test which has a much much high positive predictive value, positive predictive value of around 99 percent and that is called as NIPT non-invasive prenatal test assessing the cell free fetal DNA in mother's blood right, but I have already mentioned this is also a screening test.

So, if this is positive if this is positive then ultimately we have to go to the diagnostic test which will actually tell us whether the fetus is anomalous or not right and this diagnostic test in first trimester is by chorionic villus sampling. So, this is in nutshell, but to first understand is every woman should be counseled to go for these screening tests, but they are very costly sometimes the parents or the mother will tell that doctor I cannot afford then you can do nothing, but at least you should know counsel the importance of these tests and if they are positive then you know it needs to be further investigated and if the fetus is anomalous then you have a chance or choice of pregnancy termination right in the early weeks. So, what are the maternal risk factors for anomalous baby? Number 1 the maternal age if it is elderly female that is more than 40 years or you know 35 years of age, family history of neural tube defects or any other chromosomal defect present in the family, if there is a history in the previous pregnancy that the fetus has neural tube defect right. So, there is increased chance of recurrence what is the chance of recurrence if one previous one history of neural tube defect previous history of one neural tube defect then chance of recurrence is 5 percent, if previous two history of neural tube defect chance of recurrence is 13 percent, if previous three history of neural tube defect then chance of recurrence is 25 percent. So, there is increased chance of pregnancy you know increased chance of neural tube defect in the subsequent pregnancy and we have already discussed that this can be treated or prophylactic treatment of with folic acid 4 to 5 milligram daily right you have to give in the subsequent pregnancy right.

And if in cases that the parents are having you know they are carriers of any sex linked or autosomal traits right or one parent having a balanced translocation or Robertsonian translocation or if there is a history of recurrent miscarriage, recurrent miscarriage meaning chromosomal abnormality, mostly trisomy 16 these all will get aborted right these all will get aborted in the early trimester. So, recurrent miscarriages hint towards anomalous babies. So, every woman has a risk that her fetus may have a chromosomal abnormality. What is a priori risk before any test is being done depending upon the age of the mother and the gestational age, gestational age or period of gestation of that pregnancy we calculate a priori risk right. What is the risk that the fetus in this pregnancy may have an abnormal chromosomal assay right.

Now, next is the patient specific risk. Priori risk into likelihood ratio will give the patient specific risk and what is this likelihood ratio? This is the USG plus the biochemical parameters right biochemical parameters which are obtained in the present pregnancy by the same USG and biochemical parameters in a normal fetus right. So, we actually calculate the ratio this is the likelihood ratio on of the same measurement as the USG and the biochemical parameters which are being you know obtained in this present pregnancy we calculate that with that of a normal fetus and we will be getting the likelihood ratio and when we multiply the likelihood ratio with the priori risk we will get the patient specific risk and this patient specific risk will become the priori risk for the subsequent test. So, these are the calculations right from the findings of the

screening test we calculate the risk associated of the fetus to have any chromosomal abnormality. Now coming to the screening test the most what are the screening test that we are doing? In the first trimester I have told that we do one biochemical and one USG parameter.

What is the biochemical test? That is the dual test or dual marker right and it has two tests number one is the pap A or the pregnancy associated plasma protein A and number two is the beta HCG. These two parameters are measured in the mother's blood and this is done between 11 to 13 6 by 7 weeks of gestation right and say if we suspect or if it is a case of Down syndrome what will be the values pap A will decrease and beta HCG will increase right. So, this is the findings in case of Down syndrome if say both decreases pap A decreases beta HCG also decreases then it is a case of Edwards syndrome right. Now coming to USG marker that is the Nuchal Translucency and number three is your age. So, three parameters taken together we will assess the risk of chromosomal abnormality.

Now we will read about the Nuchal Translucency. What is this in USG? It is a USG parameter and it is screened like this say this is the fetal. So, this is the neck region of the fetus right. So, this is the neck region of the fetus this is the skin and this fluid accumulation just behind the neck of the fetus that fluid accumulation right that in USG we will see as this area this length this area this called as the Nuchal Translucency. How we measure the fetus should be in neutral position right that is the head the head of the fetus will be the head of the fetus will be in alignment with the fetal spine right and the fetus will be in you know quiescent stage there will be no extra movement of the fetus and then the head thorax the head neck and the thorax will be magnified in this view and we take this view and we measure inner border to inner border right inner border to inner border the maximum width right the maximum width and that will give the Nuchal Translucency.

Now, we will go to the theory what is Nuchal Translucency? It is measured in the midsagittal plane more conveniently the right ventral midsagittal view with the neck being in the neutral position that is neither hyperextended or nor hyper flexed right it is a neutral position in line with the fetal spine head neck and thorax are magnified fetus in quiescent stage and the distance between the fetal skin and the just behind the fetal spine right. So, it is measured from inner border to inner border in the widest plane and this is done in the when the gestational age is between 11 to 13 6 by 7 weeks it corresponds to the CRL length of 45 to 84 millimeter CRL length meaning crown rump length. So, this is the CRL length that will be around a 45 to 84 nanometer millimeter sorry right. So, in this we measure the Nuchal Translucency. Now, see here this is the Nuchal Translucency this is the maximum width from inner border to inner border.

So, here it is increased the Nuchal Translucency is increased what is normal? Normal Nuchal Translucency is up to 3 millimeter less than 3 if it is more than 3 then Nuchal Translucency is

increased and there is more chance of aneuploidies. So, NT Nuchal Translucency increase in USG that means, that it is more than or equal to 3 millimeter it occurs in cases of aneuploidies most commonly in Down's that is trisomy 21 followed by trisomy 18 that is Edward and then trisomy 13. It also occurs in Turner's syndrome that is monosomy X and also in some of the cardiac defects. So, if we get Nuchal Translucency increased in first trimester USG scan we say that it is screen positive and we go for the diagnostic test that is done by karyotyping. Karyotyping from fetal cells which are obtained from chorionic villus sampling which is done between 9 to 13 weeks of gestation right and say karyotyping is coming out to be normal that is there is no aneuploidy then there is a chance of cardiac defect.

So, we will go for fetal echocardiography to exclude any cardiac defect right. Now coming to the different parameters in the first trimester right the detection rate and the false positive rate. If only NT scan is used then the detection rate of anomalous baby is 80 percent and false positive rate is around 5 percent. If we take age dual marker that is beta HCG and pap A and also NT scan into account then the detection rate will increase to 90 percent 90 percent of the anomalous fetuses can be detected right in the first trimester and false positive rate will be 5 percent. Now if we include age, we include dual marker, we include NT scan plus we include some other first trimester USG marker that is tricuspid regurgitation, nasal bone presence and ductus venosus flow right.

These three parameters if we add or if we now also look into these parameter then we can detect around 95 percent of the anomalous babies with false positive rate being less than 3 percent right. So, these are the last three NT scan we have already laid read about. Now the other USG parameter that is the nasal bone presence right. So, how we look into the nasal bone you can see in this picture this is the nasal bone right. So, you see here these are two parallel lines right.

So, this is the tip of the nose now if you extend or go you know extend that tip of the nose behind you will see a line you know above right. So, this is the nasal skin right and just below the nasal skin are more hyper translucent right more hyper translucent line white line this is the nasal bone and if nasal bone is stressed behind it will be continuous with the frontal bone of the fetal skull. Now this nasal bone presence is an important marker if it is present then the baby is normal if it is absent then there is chance that the baby is anomalous right. So, nasal bone presence will help in detecting normal and abnormal fetus sometimes nasal bone does not get ossified in 11 weeks or beginning of 12 weeks. So, in that case we need to repeat it after 1 week right.

So, number 1 was nasal bone number 2 is tricuspid regurgitation. Tricuspid regurgitation also it is present in cases of say anomalous baby cardiac defects right cardiac defects in case of chromosomal defects it is present normally it should be absent and number 3 is ductus venosus

flow ductus venosus flow pattern. Ductus venosus actually you know connecting the umbilical arteries with the heart and carrying only oxygenated blood that we have read in fetal circulation and what will be the flow pattern it will be like this right. So, this is the A wave this A wave A wave should be present and it should be positive if A wave is absent or reversed then it is a case of anomalous baby in case of you know cardiac defects in case of your different congenital defects we get the A wave to be absent or reversed right. So, these were the USG parameters in first trimester.

Now coming to the second trimester second trimester again same biochemical parameter and USG parameter there are 2 biochemical tests that we can do in second trimester second trimester meaning 15 to 22 weeks of gestation. Number 1 is the triple test triple test contains 3 parameters that is the beta HCG alpha-fetoprotein and unconjugated estriol right and quadruple test is triple test plus inhibin A. So, all the 3 beta HCG alpha-fetoprotein unconjugated estriol taken plus inhibin A will give the quadruple test sensitivity of triple test is 70 percent whereas, quadruple test is around 85 percent right. So, this was only biochemical. Now coming to the USG parameters what are the USG parameters we look into in second trimester it is the skin fold the nuchal skin fold thickness not the nuchal translucency, but the skin fold thickness which if more than 6 millimeter it is anomalous.

Absence of nasal bone I have already talked about nasal bone presence indicates that is a normal fetus. So, nasal bone absent that means, it is abnormal right it can become abnormal short humerus short femur, simian crease you know single crease over the palm it is called as simian crease. Saddle gap saddle gap is the gap between the you know thumb and the first finger. So, this is the foot right. So, this is the foot and this is called as the saddle gap between the thumb and the first finger.

There is an increased gap between this. Echogenic cardiac focus, echogenic bowel, dilated pelvic-calecial system, choroid plexus cyst all these are markers of anomalous babies right. Now only for Down syndrome if we discuss then what are they that is nasal bone absent right. Echogenic cardiac focus, echogenic bowel, short humerus these are all hinting towards Down syndrome short femur right. Echogenic bowel, intra cardiac, intra cardiac echogenic focus. Different USG parameters which we should know and if present then we all know more in investigate for Down syndrome.

Atrioventricular septal defect, short femur, short uterus, saddle gap right, renal pelvic teses all these are features of Down syndrome. Coming to Turner syndrome it can be there is presence of cystic hygroma or ascites or pleural effusion or coarctation of aorta all these coarctation of aorta all these if noted in USG it will suggest more towards Turner syndrome. Turner syndrome is monosomy X, Down's is trisomy 21. Now coming to two other syndromes that is the Edwards syndrome, Edwards syndrome is trisomy 18 right. What are they that is you know this

polydactyly, polydactyly.

Then see this one rocker bottom feet right, then clubbed fruit, clenched feast these are all features of Edwards syndrome right. There can be choroid plexus cyst in the head, choroid plexus cyst. So, these will hint towards Edwards syndrome and lastly there is another syndrome that is the Patau syndrome which is trisomy 13. Here also we can get polydactyly right. So, this is a question polydactyly you can get cleft lip, cleft palate right.

Then absent eyebrows microcephaly or small head right. Then your holoprosencephaly are all features of Patau syndrome. Cardiac defects, neural tube behind is the spinal cord. So, neural tube defects all undescended testes all can be associated with Patau syndrome. So, these were the USG parameters. Now coming to the most important if one USG parameter if we find and what is the most important isolated USG parameter having the maximum risk of aneuploidy that is your nuchal skin fold thickness more than 6 millimeter right.

And second isolated USG parameter linked mostly with anomalous fetus is short femur sorry short femur and least risk of aneuploidy is associated with only if there is single choroid plexus cyst then there is least chance that the baby is you know anomalous. Only isolated choroid plexus cyst does not have such you know so much importance. So, these were the USG parameters. Now coming to calculation of the relative risk in India you know coming from all the biochemical tests the USG parameters, the dual marker test, the triple marker test right all these tests are taken and we they have a cut off value 1 in 250 is for Down syndrome and 1 in 100 is for Edwards syndrome. If it is more than the cut off value then the screening is positive and you go for diagnostic test right.

So, combined test I have talked about NT scan only, combined test meaning NT scan burst dual marker sometimes we also add the age that will increase the sensitivity. Now if we add all the 3 other USG parameters that is the nasal bone, the ductus venosus flow and the tricuspid regurgitation that will further increase the detection rate. And then if the screening is positive then we will go for confirmation by karyotyping by chorionic villus sampling and we collect the fetal tissue and we know note the chromosomal structure of the fetus right. And integrated screening first trimester screening plus second trimester screening this together we will got called as the integrated screening right. So, first trimester we know NT scan plus dual marker and second trimester you also know USG plus your triple, triple marker or quadruple marker right.

Now this is the integrated screening and then we calculate the risk and if second trimester screen is positive then what we will do again we go for diagnostic test that is karyotyping and here in second trimester karyotyping is done by amniocentesis, amniocentesis this is done in 16 to 18 weeks of gestation right. So, that was all regarding the screening test. In nutshell first

trimester screen that is the between 11 to 13 6 by 7 weeks of gestation go for dual marker, go for NT scan as well as nasal bone presence, your tricuspid regurgitation whether present or absent and also ductus venosus flow pattern assessment in Doppler study in USG right. That was the USG and number 2 the blood test maternal blood test for dual marker that is beta HCG and pap A right and then these 2 taken together will give you the risk assessment for down syndrome and other aneuploidies right. Now coming to second trimester we will go for the triple marker or the quadruple marker as well as the USG parameters.

What I have missed for you is the triple marker and quadruple marker what will happen? So, say this is the beta HCG, this is pap A, this is your triple marker meaning 3 right. So, alpha-fetoprotein, unconjugated estriol and inhibin A. So, total 5 parameters and here is your trisomy 21, trisomy 13 and trisomy 18. Say in trisomy 21 only beta HCG and inhibin is high just remember.

So, this will be high, this will be high, rest all will be low. In trisomy 13 only inhibin is high right. So, this will be low, this will be low, this will be low, this will be low only this will be high. In trisomy 18 all decreases. So, all the parameters will be decreased in trisomy 18.

These are the findings of triple or quadruple marker right. So, this was regarding the your first trimester and second trimester screening. So, this was all. Thank you for today's class. Keep reading, go through the textbook, go through the different questions and in the next class we will discuss the invasive test, the non-invasive test and a little bit regarding the NIPT that is cell free fetal DNA assessment in mother's blood right. So, thank you.